CHEMISTRY LETTERS, pp. 379-384, 1972. Published by the Chemical Society of Japan

SOLID PHASE PEPTIDE SYNTHESIS BY OXIDATION-REDUCTION CONDENSATION. ATTACHMENT OF VARIOUS AMINO ACIDS AND PEPTIDE FRAGMENTS TO THE HYDROXYMETHYL RESIN

Rei MATSUEDA, Eiichi KITAZAWA, Hiroshi MARUYAMA, Hidekuni TAKAHAGI, and Teruaki MUKAIYAMA*

Product Development Laboratories, Sankyo Co., Ltd.
Hiromachi Shinagawa-ku, Tokyo, Japan

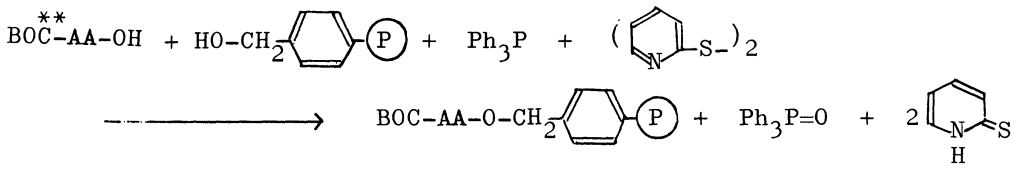
* Laboratory of Organic Chemistry, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo, Japan

An attachment of the first amino acid to the resin in solid phase method by oxidation-reduction condensation was investigated. Various amino acids and peptide fragments were attached to the hydroxymethyl resin and chain-lengthening of these aminoacyl resins with amino acids or peptide fragments was successfully achieved by the same procedure.

It was shown in earlier communications[1] that peptides and active esters of various amino acids with high optical purities were synthesized in excellent yields by oxidation-reduction condensation with the use of triphenylphosphine and 2,2'-dipyridyl disulfide. In the present experiment, an attachment of various amino acids and peptide fragments to the hydroxymethyl resin by this reaction process was investigated.

By the solid phase peptide synthesis with N,N'-dicyclohexylcarbodiimide, each coupling for peptide bond formation is carried out under mild conditions. However, the first amino acid is introduced to the resin by refluxing an amino acid and the resin at 80° for 48 hr. It seems desirable that both of the attachment of the first amino acid to the resin and subsequent couplings are carried out under mild condition by the same procedure. For this purpose, both the attachment of the first amino acid to the resin and the chain-lengthening of this aminoacyl resin with the subsequent new amino acid by the oxidation-reduction condensation was studied. First, the usual 2% crosslinked chloromethyl resin (Schwarz-Mann, New York, Cl content 2.0 mmol/g) was converted into a hydroxymethyl resin by a literature procedure[2)-a] and esterifications of this resin with various N-protected amino acids and peptide fragments were studied.

** Abbreviations: BOC, t-butyloxycarbonyl; DMF, dimethylformamide; Cys(Bzl(OMe)), S-p-methoxybenzylcysteine; TFA, trifluoroacetic acid.



In a typical experiment, 2.0 g of the hydroxymethyl resin was suspended in 20 ml of CH_2Cl_2 and shaken for 30 min in a vessel for solid phase synthesis, and the resin was washed with CH_2Cl_2. After the addition of 10 ml of CH_2Cl_2 containing 3 eq (12 mmol) each of BOC-L-phenylalanine, 2,2'-dipyridyl disulfide and shaking for a few minutes, 5 ml of CH_2Cl_2 containing 3 eq (12 mmol) of triphenylphosphine was added at room temperature. The mixture was shaken for 24 hr at room temperature and was washed with CH_2Cl_2, EtOH and DMF. From the washing solvents, 3.76 mmol of BOC-L-phenylalanine (47% yield from BOC-L-phenylalanine used) was recovered[3]. The remaining free hydroxyl groups on the resin were covered by acetylation with 10 eq (40 mmol) of acetic anhydride and triethylamine in DMF for 2 hr as described in the literature[2)-b)]. The resin was washed with DMF, EtOH and CH_2Cl_2, filtered and dried in vacuo. Fifty mg of the resin thus obtained was treated in anhydrous HF[4] and the phenylalanine liberated was quantitated on an amino acid analyzer to be 0.38 mmol/g, 6.3% yield from BOC-L-phenylalanine used and 19% yield from the hydroxyl content. The effects of molar ratio of reagents and reaction time of the above reaction are shown in Fig 1 and 2. The results indicate that the resin with desirable amino acid content of 0.1-0.5 mmol/g[2)-c)] is obtained by shaking for 24 hr with

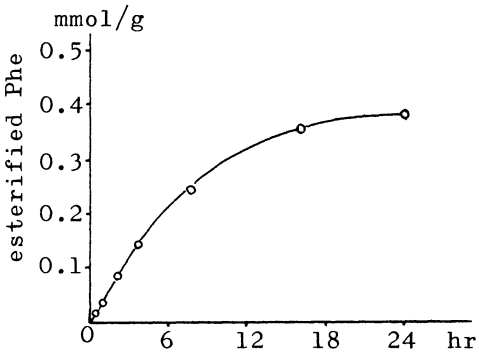


Fig. 1. Esterification rate (3 eq. of BOC-Phe-OH and reagents were used.)

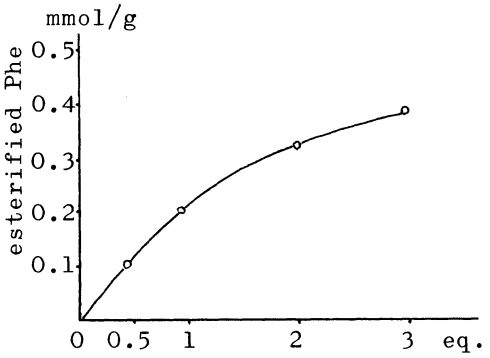


Fig. 2. Effect of quantity of reagents (24 hr reaction)

the use of 3 eq of BOC-amino acid, triphenylphosphine and 2,2'-dipyridyl disulfide. By this procedure esterification of various amino acids and peptide fragments was tried, and favorable results were obtained as summarized in Table I. N-protected amino acid hydrates and peptide fragments[5] were easily attached to the resin by

Table I

Example	Amino Acid, Peptide	Reaction Solvent	Amount Esterified[a] (mmol/g)
1.	BOC-Gly-OH	CH_2Cl_2	0.44
2.	BOC-L-Ala-OH·1/2H_2O	"	0.25
3.	BOC-L-Phe-OH	"	0.38
4.	BOC-L-Pro-OH	"	0.16
5.	BOC-L-Val-OH	"	0.12
6.	BOC-L-Ile-OH·1/2H_2O	"	0.11
7.	BOC-L-Ala-L-Ala-OH·H_2O	"	0.11 (Ala-Ala)[b]
8.	BOC-L-Lys(Z)-L-Phe-OH	"	0.26 (Lys, Phe)[b]
9.	BOC-L-Asp(NH_2)-OH	DMF	0.19 (Asp(NH_2))[c]
10.	BOC-L-Glu(NH_2)-OH	"	0.21 (Glu(NH_2))[c]
11.	BOC-L-Arg(NO_2)-OH	"	0.12
12.	BOC-L-Try-OH	"	0.12
13.	BOC-L-Cys(Bzl)-OH	CH_2Cl_2	0.18
14.	BOC-L-Met-OH	"	0.15[d]
15.	BOC-L-His(Bzl)-OH	DMF	0.44 (His(Bzl))[e]

a) In most cases, 50 mg portion was treated in anhydrous HF and the liberated amino acids were quantitated on an amino acid analyzer. In examples 8 and 15, resins were hydrolyzed in conc HCl/dioxane (1:1 v/v) for 24 hr[2)-d)] and in example 8 it was further hydrolyzed with 6 N HCl for 24 hr.

b) The value of example 7 was an amount of dipeptide of Ala-Ala and example 8 was that of Phe and Lys.

c) In example 9, a single peak of asparagine appeared on an amino acid analyzer, and aspartic acid or the other peak was not observed. The amount was calculated from an authentic asparagine. Similar results were observed also in example 10 for glutamine.

d) This value was good agreement with a sulfur analysis of the resin.

e) This was determined by the procedure as described in J. M. Stewart and J.D. Young, "Solid Phase Peptide Synthesis," W.H. Freeman, San Francisco, Calif., 1969, p 54.

this procedure. No nitrile formation and trans-esterification were observed in cases of asparagine and glutamine[6]. Favorable results were also obtained in cases of arginine, cysteine and tryptophan. Attachment of tryptophan to the resin was not previously reported. BOC-methionine[7] and BOC-im-benzylhistidine[7] were successfully attached by this procedure. This method has the following special practical merits in solid phase peptide synthesis: this procedure permits esterification of all the BOC-amino acids and peptides tested (see Table I) and it allows an attachment of a desired amount of these amino acids and peptides in a rather homogeneous environment on the resin. According to the present esterification procedure, the reaction proceeds under milder condition and in shorter reaction time than those carried out by an ordinary method. Moreover, the reaction condition and the operation are the same with the subsequent chain-lengthening acylation, and consequently the incorporation of the esterification step in the automated process is possible.

Next, chain-lengthening of these aminoacyl resin with a new amino acid or peptide by oxidation-reduction condensation was further studied. General procedure is shown in Table 2. Since triphenylphosphine oxide and 2-mercaptopyridine,

Table 2

General Procedure of Chain Elongation by Oxidation-Reduction Condensation

1. CH_2Cl_2 wash
2. Deprotection with TFA/CH_2Cl_2 (1:1 v/v) for 30 min
3. CH_2Cl_2 wash
4. $CHCl_3$ wash
5. Neutralization with $Et_3N/CHCl_3$ (1:10 v/v)
6. $CHCl_3$ wash
7. EtOH wash
8. CH_2Cl_2 or DMF wash
9. Coupling Reaction in CH_2Cl_2 or DMF using 3 eq of BOC-amino acid and reagents for 4 hr
10. CH_2Cl_2 or DMF wash

co-products in oxidation-reduction condensation, are very soluble in solvents, they are washed away thoroughly by washing procedure and solvent system is simplified than N,N'-dicyclohexylcarbodiimide-mediated coupling which accompanies insoluble urea derivatives. For example, 2.0 g of BOC-methionine-resin (0.15 mmol/g) was suspended in 20 ml of CH_2Cl_2 and was shaken for 30 min in a vessel for solid phase synthesis and the resin is washed with CH_2Cl_2. Deprotection of BOC group was undertaken with TFA/CH_2Cl_2 (1:1 v/v) for 30 min, the suspension was neutralized with $Et_3N/CHCl_3$ (1:10 v/v) and the resin was washed with $CHCl_3$ and CH_2Cl_2. After an addition of 10 ml of CH_2Cl_2 containing 3 eq (0.9 mmol) each of BOC-glycine and 2,2'-dipyridyl disulfide the reaction mixture was shaken for a few minutes. Then 5 ml of CH_2Cl_2

containing 3 eq (0.9 mmol) of triphenylphosphine was further added at room temperature. The mixture was shaken for 4 hr at room temperature and was washed with CH_2Cl_2, EtOH, filtered and dried *in vacuo*. A portion of the resin was hydrolyzed in 6N HCl at 110° for 24 hr and the amino acid analysis of the hydrolysate gave the expected amino acid ratio: Met, 1; Gly, 1.03. In a similar way, several aminoacyl resins were coupled successfully with amino acids or peptide fragments as shown in Table 3. Example 3 indicates that BOC-Ala-Ala-O-resin attached as dipeptide was chain-lengthened quantitatively by the succeeding stepwise coupling.

Table 3

Example	Chain-Lengthened Peptide Resin[a)]	Amino Acid Ratios[b)]
1.	BOC-Gly-{ Met-(P)	Gly, 1.03; Met, 1.
2.	BOC-Ser(Bzl)-Tyr(Bzl)-Gly-Arg(NO_2)-Pro-{ Gly-(P)	Ser, 0.96; Tyr, 0.95; Gly, 2.11; Arg, 1.05; Pro, 1.
3.	BOC-Asp(OBzl)-Ser(Bzl)-Thr(Bzl)-Ser(Bzl)-{ Ala-Ala-(P)	Asp, 1; Ser 1.97; Thr, 0.98; Ala, 2.08.
4.	BOC-Cys(Bzl(OMe))[c)]-Phe-| Phe-Glu(NH_2)-Asp(NH_2)[c)]-| Cys(Bzl(OMe))-Pro-Lys(Z)-{Gly-(P)	Cys, 0.97; Phe, 2.03; Asp, 1.03; Glu, 1; Pro, 1.00; Lys, 0.95; Gly, 1.04; NH_3, 2.15.

a) A symbol of { line indicates the initial point of chain-lengthening and | line indicates the point of coupling. A symbol of ← shows the length of peptide chain once coupled.

b) A portion of peptide resin was hydrolyzed in 6N HCl for 24 hr and amino acid ratio was determined on amino acid analyzer. In example 2 and 3, the peptide resin was treated in HF before hydrolysis.

c) Acetylation with 10 eq of acetic anhydride and triethylamine was undertaken at these points.

Example 4 is an example of elongation of peptide chain by fragment condensation on the resin. Coupling between BOC-L-Phe-L-Glu(NH_2)-L-Asp(NH_2)-OH and H-L-Cys(Bzl(OMe))-L-Pro-Lys(Z)-Gly-O-resin was carried out in DMF at -15°C for 10 hr in the presence of 6 eq of 2-mercaptopyridine and subsequent coupling of BOC-L-Cys(Bzl(OMe))-L-Phe-OH with the resulting peptide-resin was undertaken in CH_2Cl_2 at room temperature for 8 hr. These results show that chain-lengthening acylations by oxidation-reduction condensation was effectively carried out in solid phase peptide synthesis.

In conclusion, it is noted that oxidation-reduction condensation reaction can

be successfully applied to the esterification of the first amino acids or peptides to the resin under a mild condition, and peptide chain elongation of a stepwise strategy or a fragment condensation are achieved by the same operation.

REFERENCES

1) T. Mukaiyama, R. Matsueda and H. Maruyama, Bull. Chem. Soc. Japan, 43, 1271 (1970).
T. Mukaiyama, R. Matsueda and M. Suzuki, Tetrahedron Lett., 1901 (1970).
T. Mukaiyama, K. Goto, R. Matsueda and M. Ueki, Tetrahedron Lett., 5293 (1970).

2)-a) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969, p 27.
-b) ibid, p 33
-c) ibid, p 32
-d) ibid, p 53

3) Solvents were removed and the residue was stirred for 30 min after the addition of 50 ml of water containing 1% acetic acid. From the resulting mixture, BOC-phenylalanine was extracted with ethyl acetate. It was back-extracted with 5% $NaHCO_3$ and reextracted with ethyl acetate after acidification to pH 3. From the extract, BOC-phenylalanine with mp 81-4°C was recovered.

4) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and H. Sugihara, Bull. Chem. Soc. Japan, 40, 2164 (1967).

5) Peptide fragments are reported to be difficult to be attached by the ordinary method. H. Yajima, H. Kawatani and H. Watanabe, Chem. Pharm. Bull., 18, 1333 (1970).

6) Marglin has recently proposed an estefification of chloromethyl resin in DMF at room temperature to these amino acids. A. Marglin, Tetrahedron Lett., 3145 (1971).

7) These amino acids were linked by N,N'-carbonyldiimidazole or N,N'-dicyclohexylcarbodiimide condensation with hydroxymethyl resin because of the side reaction of alkylation by the ordinary method and this procedure is reported to be the only one for esterification of BOC-methionine[2)-b)].

(Received March 23, 1972)